

IN THE SPOTLIGHT

Cracking the Code of Resistance across Multiple Lines of ALK Inhibitor Therapy in Lung Cancer

Huan Qiao¹ and Christine M. Lovly^{1,2,3}

Summary: In the setting of recent exciting clinical results and numerous ongoing trials, Gainor and colleagues explored mechanisms of acquired resistance to first- and second-generation ALK inhibitors in ALK-rearranged non-small cell lung cancer and found that an increased frequency and distinct spectrums of resistance mutations emerged with the more potent second-generation inhibitors. Their findings have important and immediate clinical implications as the resistance mutations detected impart differential sensitivities to available ALK inhibitors, thereby highlighting the need for sequential biopsies with molecular testing to determine the most effective treatment strategy upon disease progression. *Cancer Discov*; 6(10); 1084-6. ©2016 AACR.

See related article by Gainor et al., p. 1118 (3).

Chromosomal rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene have been identified in a broad spectrum of malignancies, including non-small cell lung cancer (NSCLC). The rapid pace from the 2007 discovery of *ALK* rearrangements in lung cancer (1) to the 2011 approval of the first-in-class ALK tyrosine kinase inhibitor (TKI) crizotinib by the FDA has been heralded as one of the major successes in translational cancer research during the past decade. Numerous randomized clinical trials have documented the efficacy of crizotinib in patients whose lung tumors harbor *ALK* rearrangements (2), and ALK testing is now considered standard of care for patients with advanced non-squamous NSCLC.

Unfortunately, most patients whose disease initially responds to crizotinib eventually develop progressive disease, a concept referred to clinically as “acquired resistance.” Molecular mechanisms leading to crizotinib resistance include point mutations within the ALK tyrosine kinase domain that alter drug efficacy, amplification of the *ALK* fusion gene, and activation of “bypass” signaling pathways (Fig. 1; ref. 2).

More recently, second- and third-generation inhibitors with increased potency against ALK have been developed. These inhibitors have activity *in vitro* against the ALK kinase domain mutations associated with crizotinib resistance, and clinical trials have demonstrated impressive activity of these agents in patients with crizotinib-refractory ALK⁺ lung cancer (2). Corroborating the rapid pace of discovery and drug

development, three second-generation ALK inhibitors have already received FDA approval (ceritinib and alectinib) or breakthrough-therapy designation (brigatinib). Yet, acquired resistance remains a problem, even with these more potent ALK inhibitors. There is an urgent clinical need to define resistance mechanisms across multiple lines of ALK TKI therapy and to develop innovative approaches to overcome or delay drug resistance.

In this issue of *Cancer Discovery*, Gainor and colleagues present the largest systematic analysis of ALK TKI-resistant tumor samples to date. They successfully captured 103 repeat biopsy samples from 83 patients with ALK⁺ lung cancer progressing on various first- and second-generation ALK inhibitors, including crizotinib, ceritinib, alectinib, and brigatinib (3). They also developed six ceritinib-resistant, patient-derived cell lines for additional analysis. Using a combination of genetic and cell-based assays, the authors use their robust sample collection to evaluate resistance mechanisms across multiple lines of ALK TKI therapy.

First, the authors analyzed 55 tumor specimens from 51 patients with crizotinib resistance. Consistent with previous reports, they found ALK kinase domain mutations in 20% (11/55) of these specimens. The two most common mutations were L1196M (the “gatekeeper” mutation) in 7% and G1269A in 4% of patients. By contrast, *ALK* resistance mutations were present in over 50% of patients progressing on second-generation ALK inhibitors. This increased mutation frequency is thought to be reflective of the greater on-target efficacy and selectivity of these agents. Specifically, ALK kinase domain mutations were detected in 54% (13/24) of ceritinib-resistant specimens, 53% (9/17) of alectinib-resistant samples, and 71% (5/7) of brigatinib-resistant samples. Interestingly, there were somewhat distinct spectrums of mutations that were found for each of the three second-generation ALK inhibitors analyzed. For example, although an F1174 mutation was detected in 17% (4/24) of ceritinib-resistant samples, this mutation was not detected in alectinib- or brigatinib-resistant samples. Analogously, I1171 and V1180 mutations were enriched in alectinib-resistant samples, and despite the small sample size, D1203, S1206, and E1210

¹Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee. ²Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee. ³Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, Tennessee.

Corresponding Author: Christine M. Lovly, Vanderbilt-Ingram Cancer Center, 2220 Pierce Avenue, 777 Preston Research Building, Nashville, TN 37232. Phone: 615-936-3457; Fax: 615-343-2973; E-mail: christine.lovly@vanderbilt.edu

doi: 10.1158/2159-8290.CD-16-0910

©2016 American Association for Cancer Research.

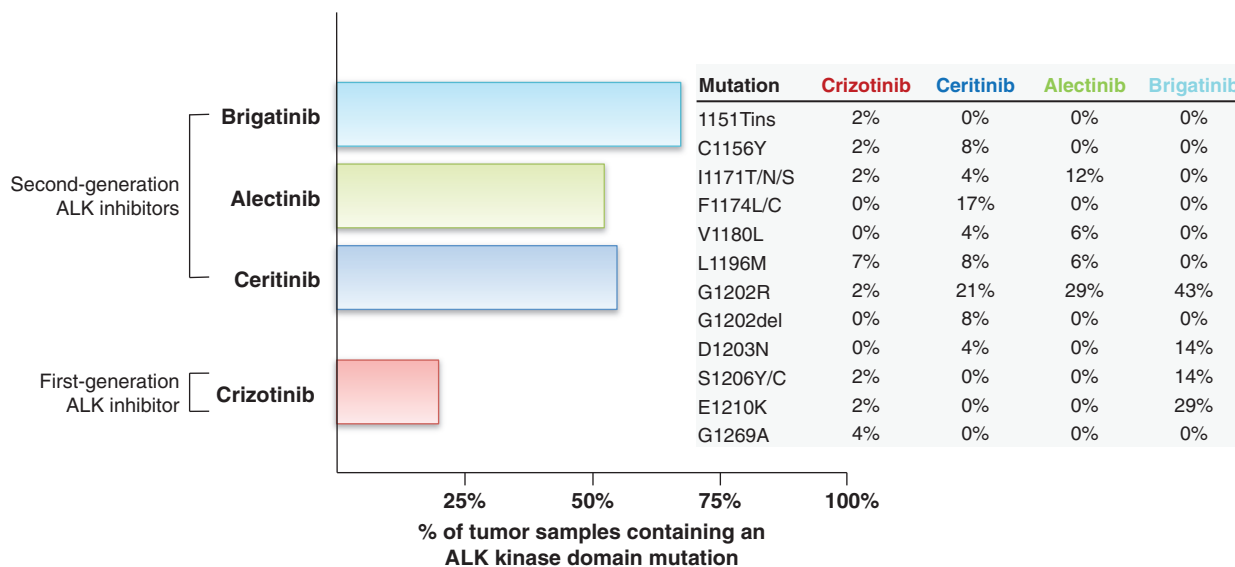


Figure 1. Summary of ALK kinase domain mutations found in samples with acquired resistance to first- and second-generation ALK TKIs. Crizotinib is a first-generation ALK TKI. Ceritinib, alectinib, and brigatinib are second-generation ALK TKIs. Only those ALK TKIs evaluated in the article by Gainor and colleagues (ref. 3) were included in this figure.

mutations were enriched in brigatinib-resistant samples. The common thread among ceritinib-, alectinib-, and brigatinib-resistant samples was the presence of the G1202R solvent front mutation in 21%, 29%, and 43% of the samples, respectively. This mutation is particularly noteworthy as it has been shown to confer high levels of resistance to most ALK TKIs, but potentially can be overcome by the third-generation ALK inhibitor lorlatinib (4). Adding another level of complexity to these data, the authors also identified compound mutations in several tumor samples. Specifically, in an analysis of biopsy samples from patients progressing on second-generation ALK TKIs, 12.5% (6/48) of the samples contained two or more concurrent resistance mutations.

Despite the increased frequency of ALK kinase domain mutations observed in patients who progressed on ceritinib, alectinib, and brigatinib, there was still a large percentage of patients whose tumors did not harbor these resistance mutations. Furthermore, there were three patients whose tumors harbored the L1196M mutation at the time of progression on ceritinib or alectinib therapy. Because previous *in vitro* studies have demonstrated that both of these agents have activity against L1196M, the authors hypothesized that other resistance mechanisms must be at play. Using immunohistochemistry on tumor samples, they show that epithelial-to-mesenchymal transition (EMT), defined by gain of vimentin expression and loss of E-cadherin expression, was observed in 42% (5/12) of cases evaluated. An important caveat of this analysis is that post-crizotinib/pre-ceritinib biopsies were not available for any of the five cases analyzed; the comparator was the diagnostic biopsy samples. Notably, EMT was detected in specimens both with and without ALK kinase domain mutations, suggesting that multiple mechanisms of resistance can occur simultaneously.

With respect to the next line of therapy after progression on a second-generation ALK inhibitor, the authors show

that the third-generation ALK inhibitor lorlatinib (4, 5) has activity against the ALK resistance mutations that develop on these agents. *In vitro*, lorlatinib was also the only agent to retain potency against the compound mutations evaluated (D1203N + E1210K and D1203N + F1174C). By contrast, lorlatinib was not effective at inhibiting the growth of three distinct ceritinib-resistant patient-derived cell lines that did not harbor ALK kinase domain mutations, suggesting that these samples may have lost ALK dependency.

This new contribution to the field of ALK⁺ lung cancer offers several novelties. Previous studies of ALK TKI resistance have been limited to small cohorts, one drug, or case reports. The strength in this study is the large sample size analyzed across numerous different ALK inhibitors. In addition, the authors provide documentation that ALK kinase domain mutations are detected in higher proportions of patients with acquired resistance to ceritinib, alectinib, and brigatinib, and that the highly resistant G1202R mutation increases with frequency in patients with acquired resistance to these agents. This is an important observation, as the presence of the G1202R mutation has implications for the next line of ALK TKI therapy. Finally, these studies show the complex evolution of resistance mutations over multiple lines of ALK TKI therapy and reinforce the need for repeat biopsies to tailor therapeutic selection.

These new findings also raise several interesting questions for future study. First, do ALK kinase domain mutations exist prior to the development of resistance or are they induced by ALK TKI therapy? Due to the lack of availability of pre-crizotinib and/or pre-second-generation ALK TKI tissue for analysis in some cases, the authors were unable to clarify this question. Recent studies have addressed this question for the EGFR^{T790M} resistance mutation with results showing that T790M can occur either by selection of preexisting T790M-positive clones or via genetic evolution of T790M-negative

Downloaded from <http://aacrjournals.org/cancerdiscovery/article-pdf/6/10/1084/1823478/1084.pdf> by guest on 22 May 2024

drug-tolerant clones (6). Notably, the mode of acquisition of T790M influenced efficacy of the next line of EGFR-directed therapy. Future work will determine if these observations hold true for ALK as well.

Second, with multiple agents now available, what is the optimal sequence of ALK TKI therapy? Will response rates and resistance mechanisms change based on the line of therapy in which a specific ALK TKI is received? The proposed NCI ALK Master Protocol will hopefully address some of these questions. This pending study is intended to evaluate second-generation ALK inhibitors with a single shared control arm, crizotinib, in patients with metastatic ALK⁺ lung cancer who have not received previous ALK TKI therapy.

Third, what additional genomic and nongenomic mechanisms are mediating resistance in those ~50% of cases which lack an ALK kinase domain mutation at the time of resistance to second-generation inhibitors? In this study, *TP53* mutations were identified in 9 (33%) tumor samples from patients who did not have ALK kinase domain mutations, but no other genetic alterations were common among the samples analyzed. Furthermore, activation of several bypass tracks, such as EGFR (7) and IGF1R (8) signaling, has been reported, but the mechanism for activation of these bypass tracks remains to be defined.

Fourth, what therapeutic options exist for patients whose tumors do not have ALK kinase domain mutations? How do we address the heterogeneity of resistance mutations in a given sample, such as the patient reported in this paper whose tumor harbored the L1196M mutation and also displayed an EMT phenotype?

Fifth, what are the molecular mechanisms of lorlatinib resistance? Will the ALK kinase domain mutations differ after progression on lorlatinib compared with progression on a second-generation ALK inhibitor? A recent case report from these authors described the appearance of an L1198 mutation at the time of lorlatinib resistance. L1198 was targeted for lorlatinib's design to increase selectivity, and this mutation can paradoxically be overcome by crizotinib (5).

Finally, are there other ways to therapeutically target ALK? For example, because oligomerization of EML4-ALK is essential for the fusion protein's transforming ability (1), can small-molecule inhibitors that inhibit oligomerization be developed as a complementary approach to inhibit ALK? Such an approach has been attempted for the BCR-ABL fusion protein (9). Furthermore, following the paradigm of EGFR inhibitors, can mutant-specific ALK inhibitors be developed to specifically target the most recalcitrant kinase domain mutations, such as G1202R?

Overall, these studies are timely and important given the current landscape of ALK inhibitors. Indeed, it is becoming increasingly common for patients to be treated with multiple lines of ALK TKI therapy. These studies show us that it is not sufficient to empirically treat patients with sequential ALK

TKI therapy, but rather, that in order to select the most efficacious next line of ALK inhibition, repeat biopsy with molecular profiling of the tumor tissue must be obtained in order to understand the resistance mechanism and tailor the therapy appropriately. In summary, the new data presented in this paper are expected to have immediate implications in the care of patients with ALK⁺ lung cancer and will inform future use of ALK inhibitors in the numerous other ALK⁺ malignancies.

Disclosure of Potential Conflicts of Interest

C.M. Lovly reports receiving commercial research grants from Astra-Zeneca and Novartis, has received speakers bureau honoraria from Qiagen and Abbott Molecular, and is a consultant/advisory board member for Pfizer, Novartis, Sequenom, Genoptix, Ariad, and Clovis. No potential conflicts of interest were disclosed by the other author.

Grant Support

H. Qiao and C.M. Lovly were supported through a LUNGevity Foundation Career Development Award and a Damon Runyon Clinical Investigator Award. C.M. Lovly was additionally supported by a V Foundation Scholar-in-Training Award, an AACR-Genentech Career Development Award, and the National Institutes of Health (NIH) and National Cancer Institute (NCI) R01-CA121210 and P01-CA129243.

Published online October 3, 2016.

REFERENCES

- Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561-6.
- Katayama R, Lovly CM, Shaw AT. Therapeutic targeting of anaplastic lymphoma kinase in lung cancer: a paradigm for precision cancer medicine. *Clin Cancer Res* 2015;21:2227-35.
- Gainor JF, Dardaei L, Yoda S, Friboulet L, Leshchiner I, Katayama R, et al. Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung cancer. *Cancer Discov* 2016;6:1118-33.
- Zou HY, Friboulet L, Kodack DP, Engstrom LD, Li Q, West M, et al. PF-06463922, an ALK/ROS1 inhibitor, overcomes resistance to first and second generation ALK Inhibitors in Preclinical Models. *Cancer Cell* 2015;28:70-81.
- Shaw AT, Friboulet L, Leshchiner I, Gainor JF, Bergqvist S, Brooun A, et al. Resensitization to crizotinib by the lorlatinib ALK resistance mutation L1198F. *N Engl J Med* 2016;374:54-61.
- Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Mulvey HE, et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med* 2016;22:262-9.
- Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med* 2012;4:120ra17.
- Lovly CM, McDonald NT, Chen H, Ortiz-Cuaran S, Heukamp LC, Yan Y, et al. Rationale for co-targeting IGF-1R and ALK in ALK fusion-positive lung cancer. *Nat Med* 2014;20:1027-34.
- Miller GD, Woessner DW, Sirch MJ, Lim CS. Multidomain targeting of Bcr-Abl by disruption of oligomerization and tyrosine kinase inhibition: toward eradication of CML. *Mol Pharmaceutics* 2013;10:3475-83.